vi) amplifying the DNA fragments by using a DNA polymerase and a primer which can combine complementarily to a residual sequence from the adapters.

9. (Amended) The process according to Claim 3, wherein the hairpin loop is eliminated by using alkaline solution.

10. (Amended) The process according to Claim 3, wherein the hairpin loop is eliminated by using RNase.

11. (Amended) The process according to Claim 3, wherein the hairpin loop is eliminated by using single strand specific exonuclease.

REMARKS

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and the following commentary.

I. Introduction

Upon entry of the present amendment, claims 1-3 and 5-12 will be pending in the instant application. Applicants have amended claims 1 and 3 to prescribe the claimed invention more definitely. In particular, claims 1 and 3 have been revised to make it clear that a hairpin structure of the adaptors is eliminated in DNA fragments to which the hairpin structure-adaptors are ligated. Additionally, claim 1 has been revised to incorporate the subject matter of cancelled claim 4. Amended claims 9-11 recite a revised dependency, in view of the cancellation of claim 4.

II. Rejection under 35 U.S.C. §103

The examiner has rejected claims 1, 3, and 7-12 over U.S. patent No. 5,270,724 to Ahern ("Ahern") in view of Sambrook *et al.* The examiner further has rejected claims 5 and 6 over Ahern, Sambrook *et al.* and U.S. patent No. 5,508,169 to Deugau *et al.* Applicants respectfully traverse these rejections.

In rejecting these claims, the examiner alleges that "it would have been *prima facie* obvious to one skilled in the art to modify a method of preparing and amplifying DNA fragments using adapters as taught by Ahern with the method of removing unligated secondary structures as taught by Sambrook *et al.* to achieve expected

advantage of developing a method for preparing and amplifying DNA fragments using hairpin loop adapters." Thus, the examiner is heard to assert that Ahern and Sambrook together evidence motivation in the art to have used hairpin structures and to have effected removal of single-stranded loop portions, and that such motivation is sufficient to render the claimed invention obvious. Applicants submit, however, that the examiner has overlooked or misapprehended important differences separating the claimed methodology from the method of Ahern.

The cited reference discloses a methodology, termed "Boomerang DNA Amplification" (BDA), that is an alternative to the Polymerase Chain Reaction ("PCR") for amplifying a DNA sequence of interest ("SOI"). According to Ahern, PCR has certain drawbacks, *e.g.*, that replication from each primer must proceed in the direction of the primer on the complementary strand. Accordingly, PCR can accommodate amplification only for sequences located between primer target sequences, even though it often is necessary or desirable to amplify sequences located outside a region flanked by primer target sequences. Another disadvantage of PCR is that it entails the use of two primers, which necessitates a detailed knowledge of sequences found in two separate regions near the sequence of interest. This information is not always available or readily obtainable. *See* column 1, line 56 – column 2, line 2.

Thus, Ahern states that a key advantage of BDA is that DNA amplification can be performed using only one primer. As a result, the DNA that is amplified via BDA is not limited to a region of the DNA situated between two primers. By the same token, BDA requires information on the sequence of at least a portion of the primer target site, in order that an appropriate primer that is homologous to the primer target site can be prepared for use in BDA.

Unlike Ahern, the claimed method does not need any information on sequences of DNA to be amplified, including a sequence of a primer target site in DNA. Instead, the claimed method uses, as a primer, an adaptor in a hairpin structure. That is, after ligating hairpin-structure adaptors to DNA fragments and removing excessive adaptors, the claimed method eliminates a hairpin structure of the adaptors, *i.e.*, opens the hairpin structure of the adaptors by using, for example, an alkaline solution, an Rnase, or a single strand-specific exonuclease. Then, the DNA fragments containing the adaptors without hairpin structure are amplified, employing an opened adaptor as a primer. That

is, hairpin-structure adaptors ligated to DNA fragments are opened before the amplification of DNA fragments.

By contrast, the adaptors of Ahern are single-strand or double-strand polynucleotides having internal sequences that are complementary to each other and that are capable of annealing to each other, forming a duplex, under appropriate conditions. In BDA, because only one primer sequence for one of double strands is available, Ahern's adaptors are required to connect ends of double strands of DNA fragments, to form a closed loop structure. As a result, a subsequent DNA replication (primer extension) is allowed to proceed for a time sufficient for the DNA polymerase molecules to circumnavigate fully the close-loop structure that contains the SOI. Accordingly, the hairpin structure of the adaptors must not be opened or eliminated before the replication or amplification of DNA fragments. Moreover, a hairpin structure is replicated or amplified with the SOI. A single-stranded portion of the DNAs, in particular, a closed-loop structure lacking the SOI, is degraded only after replication or amplification, either using Centricon-100 filters or single-strand-specific nucleases.

It is apparent, therefore, that the claimed method is differentiated from Aherns' BDA, in that the former uses hairpin-structure adaptors as a primer, not as a connector of double strands of DNA fragments, which means that the hairpin structure opens before replication or amplification of DNA fragments. To highlight these differences, applicants have amended claims 1 and 3 in the manner explained above.

Neither Sambrook *et al.* nor Deugau *et al.* cures the deficiencies of Ahern. The relevant teachings of Sambrook are limited to a technique for removing hairpin structures by means of an alkaline solution and treatment with Rnase and with exonulcease III. Deugau *et al.* only teaches a method that uses type IIs and IIip for digesting a DNA fragment to produce fragments with cohesive ends. None of these references teaches or suggests using hairpin-structure adaptors as a primer and opening the adaptors before replication or amplification of DNA fragments.

Thus, no reasonable combination of the cited references evidences a motivation somehow to adapt Ahern's methodology to use hairpin-structure adaptors as a primer and to open such a structure before DNA replication or amplification. Even if there were such motivation, the combined teachings of the cited publications would not have led the skilled artisan to applicants' claimed method. Accordingly, there is no *prima facie case* of obviousness.

Applicants respectfully request, therefore, that the Examiner withdraw the pending obviousness rejection. Applicants also believe that the present application is in a condition for allowance. Favorable reconsideration of the application as amended is respectfully requested. The examiner is invited to contact the undersigned by telephone, if he feels that a telephone interview could advance prosecution.

Respectfully submitted,

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VERSIONS WITH MARKING TO SHOW CHANGES MADE

Marked up rewritten claims:

1. (Amended). A process for preparing a library of DNA fragments of which terminal sequences are known by using a DNA of which base sequence is completely unidentified, which comprises:

i) digesting a DNA into fragments which have single-strand cohesive ends by using a restriction enzyme,

ii) preparing a series of hairpin loop adapters which have single-strand cohesive ends of which base sequence is known;

iii) ligating the DNA fragments with the hairpin loop adapters prepared in the above step ii) by using a DNA ligase; and

iv) eliminating [the] a hair pin loop structure only from the DNA fragments which contain the hairpin loop adapters, obtained in step iii), by using an alkaline solution, an RNase or a single strand specific exonuclease.

3. (Amended) A process for selective amplifying DNA of which base sequence is completely unidentified, which comprises:

i) digesting a DNA into fragments which have a single-strand cohesive end group by using a restriction enzyme,

ii) preparing hairpin loop adaptors which have the single-strand cohesive end which can be complementarily combined to and ligated on the both ends of the DNA fragments obtained in step i);

iii) ligating the DNA fragments with the hairpin loop adapters thus prepared by using a DNA ligase;

iv) removing DNA fragments and hairpin loop adapters which have not participated in the ligation reaction by using an exonuclease; [and]

v) eliminating a hairpin loop structure from the DNA fragments on which said hairpin loop adapters are ligated in step iii); and

[v] vi amplifying the DNA fragments by using a DNA polymerase and a primer which can combine complementarily to a residual sequence from the adapters.

9. (Amended) The process according to Claim [4] 3, wherein the hairpin loop is eliminated by using alkaline solution.

10. (Amended) The process according to Claim [4] 3, wherein the hairpin loop is eliminated by using RNase.

11. (Amended) The process according to Claim [4] 3, wherein the hairpin loop is eliminated by using single strand specific exonuclease.